FIG. 1

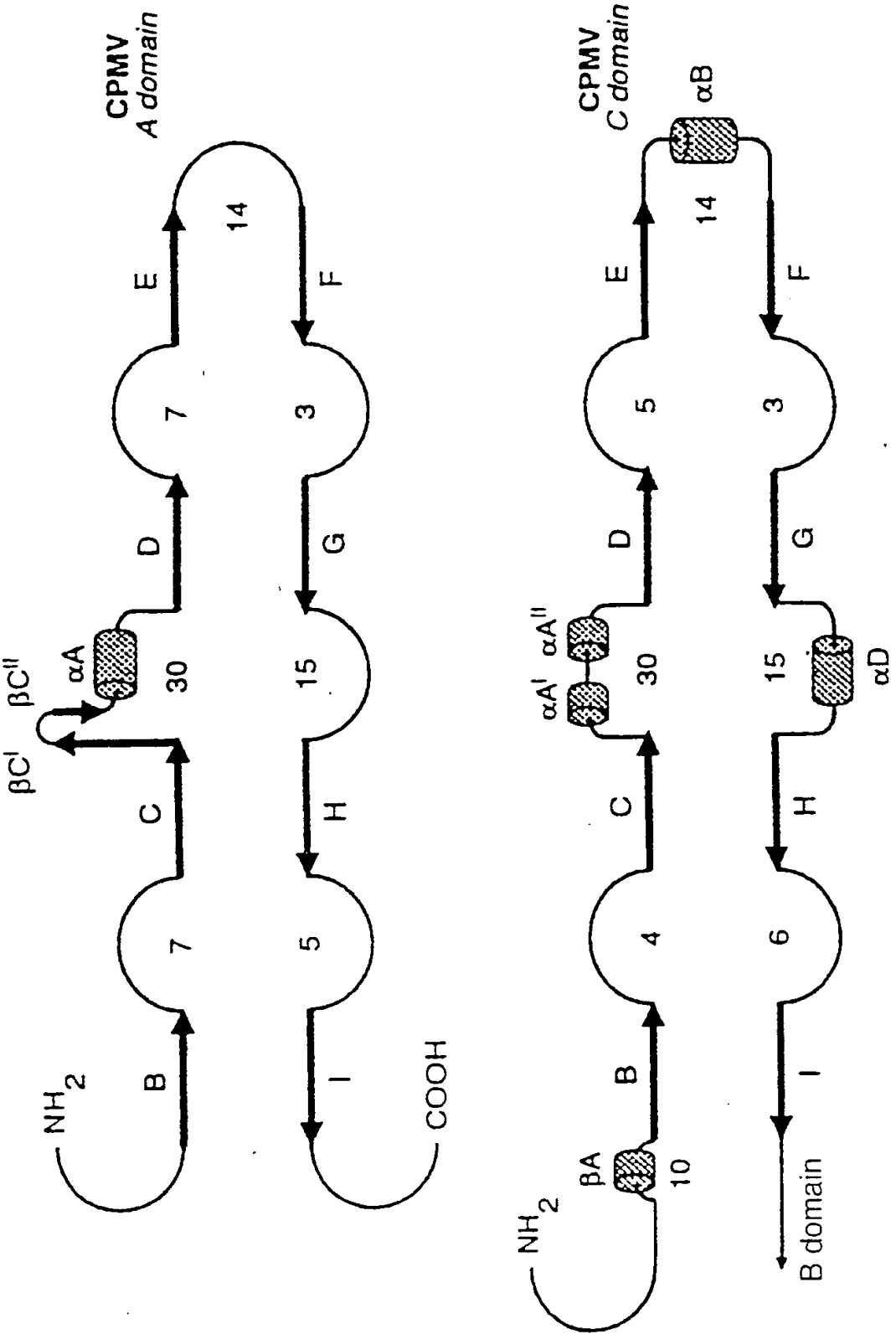
CPMV
A domain
NH2
B
7
C
βC'
βC''
αA
30
D
7
E
14
F
3
G
15
H
5
I
COOH
CPMV
C domain
αB
14
E
5
F
3
D
αA'
αA''
30
G
15
αD
C
4
H
6
βA
10
B
I
NH2
B domain

FIG. 1(contd.)

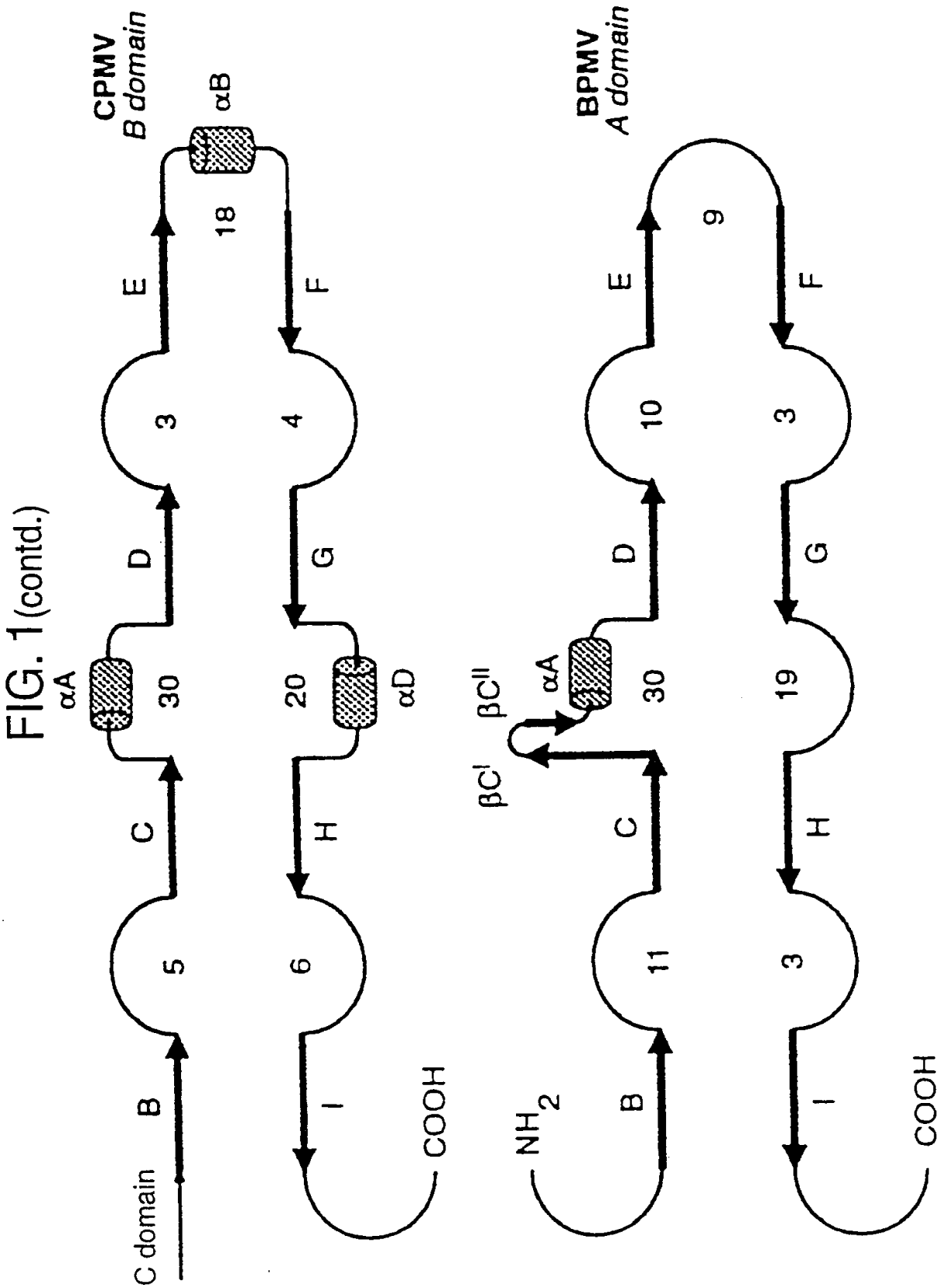
CPMV
B domain
αB
αA
αD
C domain
B
5
C
30
D
3
E
18
F
4
G
20
H
6
I
COOH
BPMV
A domain
αA
βC'
βC''
NH2
B
11
C
30
D
10
E
9
F
3
G
19
H
3
I
COOH

FIG. 1(contd.)

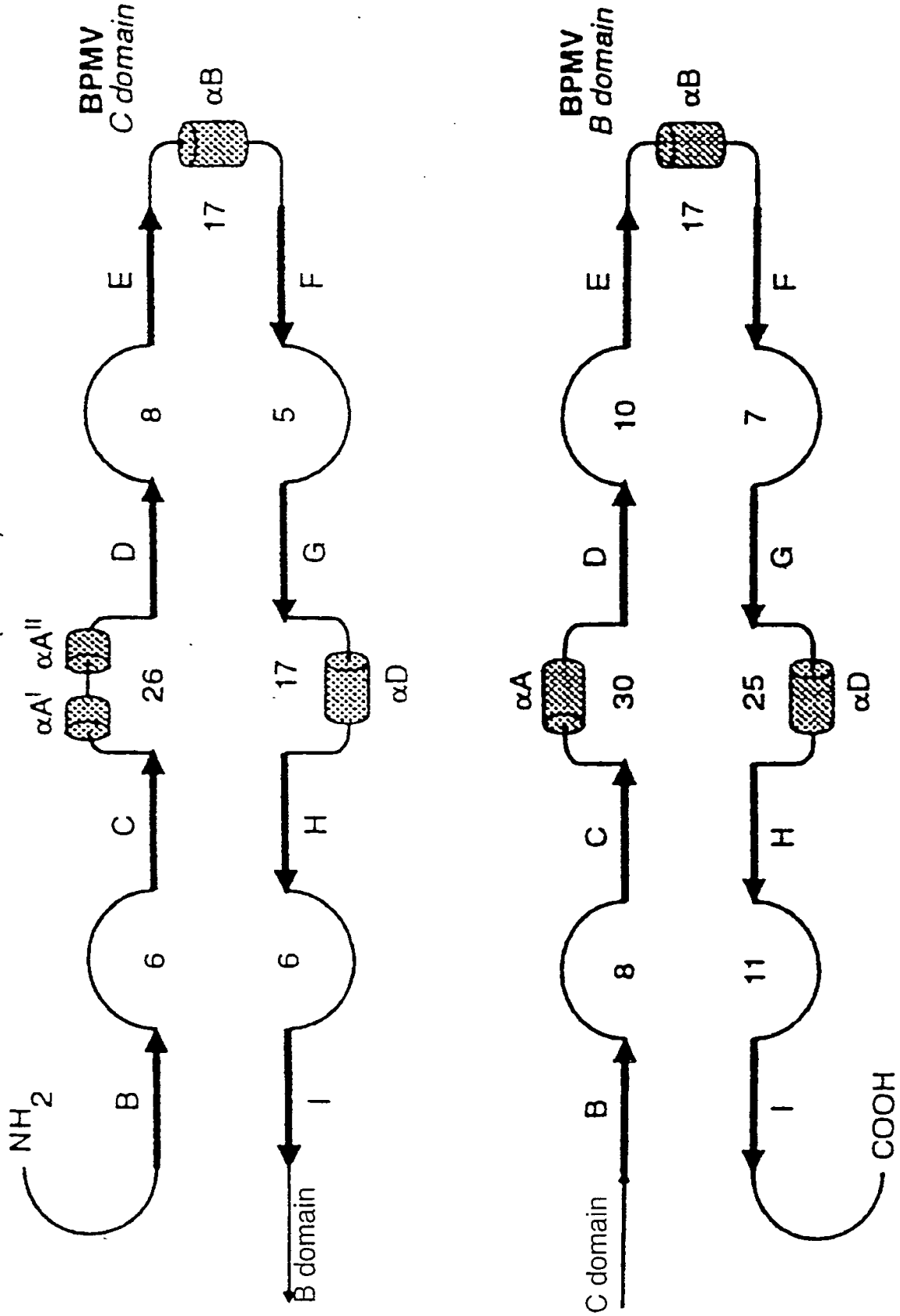
BPMV
C domain
αB
17
E
F
8
5
D
G
αA' αA''
26
17
αD
C
H
6
6
NH2
B
I
B domain
BPMV
B domain
αB
17
E
F
10
7
D
G
αA
30
25
αD
C
H
8
11
B
I
COOH
C domain

FIG. 1(contd.)

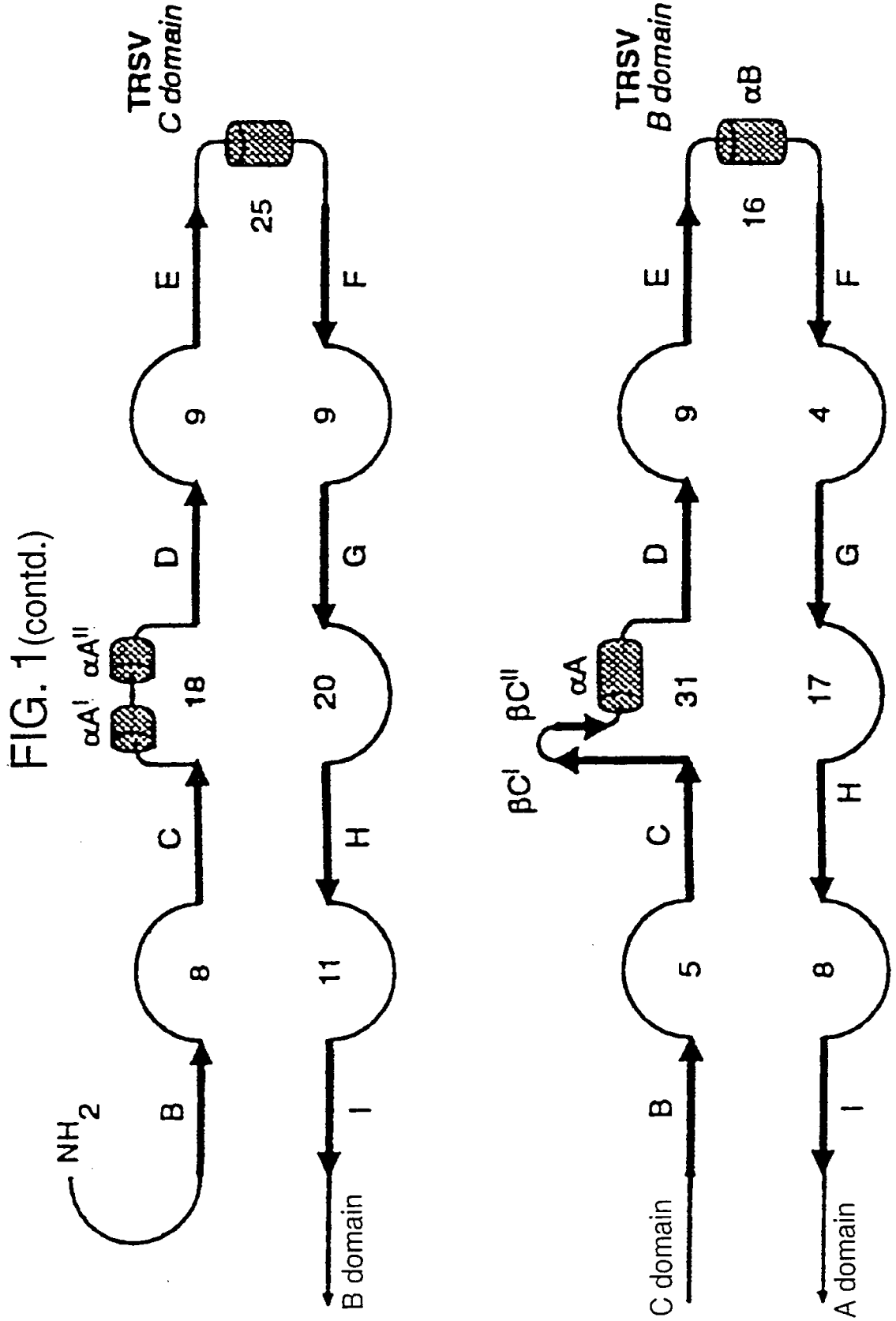
TRSV
C domain
TRSV
B domain
αB
αA' αA''
αA
βC' βC''
NH2
B
C
D
E
F
G
H
I
8
18
9
25
9
20
11
31
5
16
4
17
8
B domain
C domain
A domain

FIG. 1 (contd.)

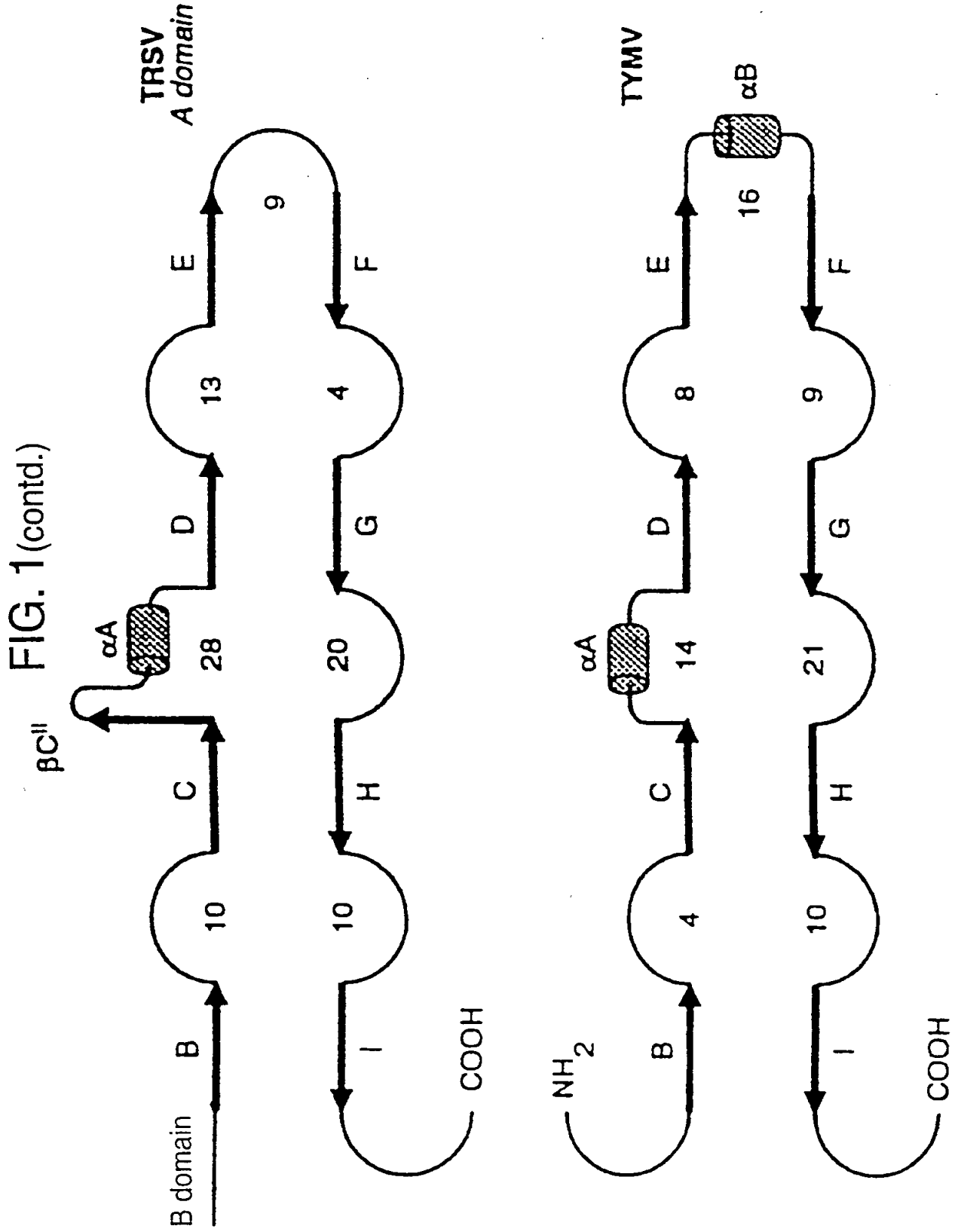
TRSV
A domain
TYMV
αB
αA
βC''
B domain
B
C
D
E
F
G
H
I
COOH
NH2
10
28
13
9
4
20
10
14
8
16
21

FIG. 1(contd.)

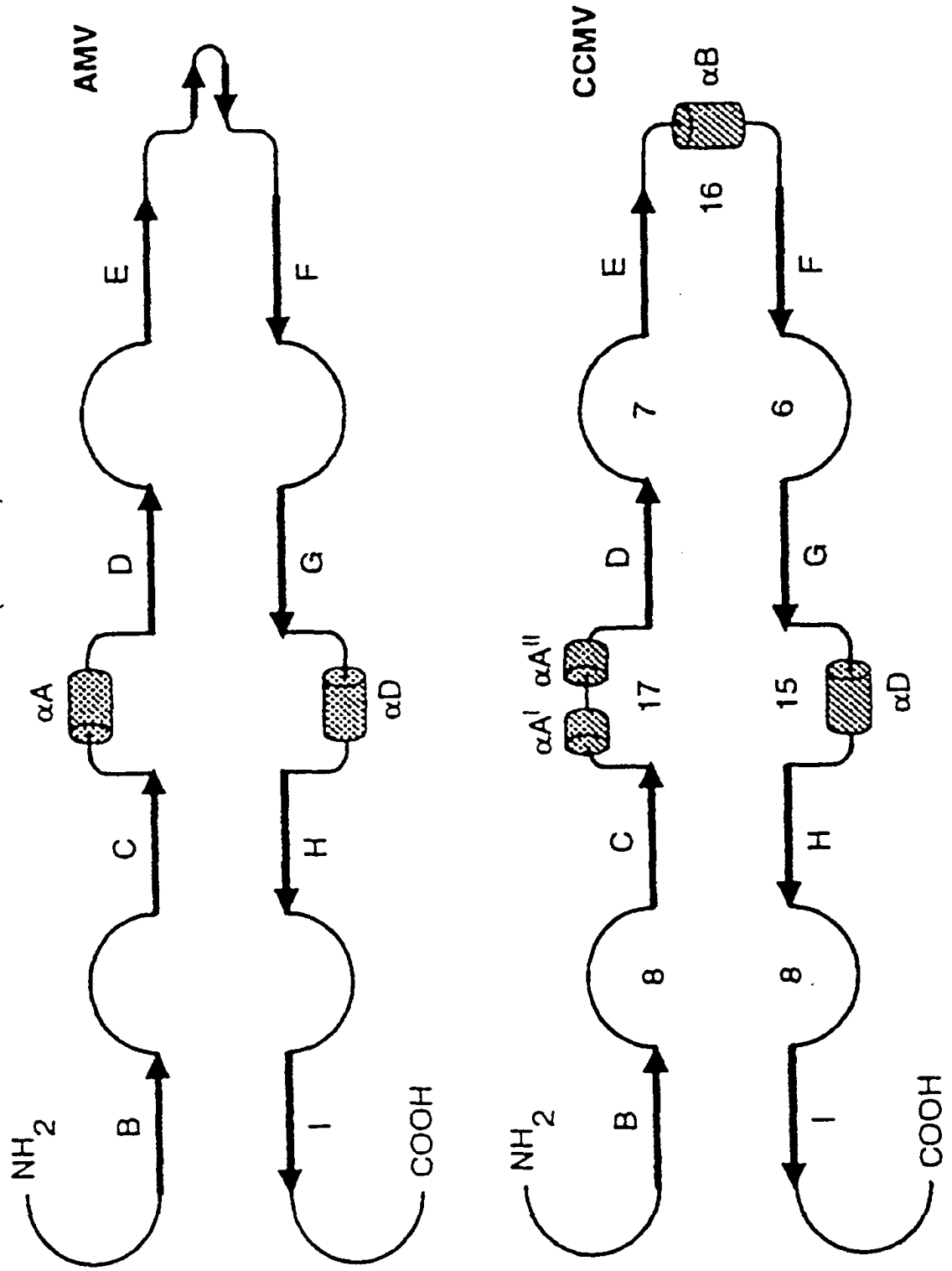

FIG. 1(contd.)

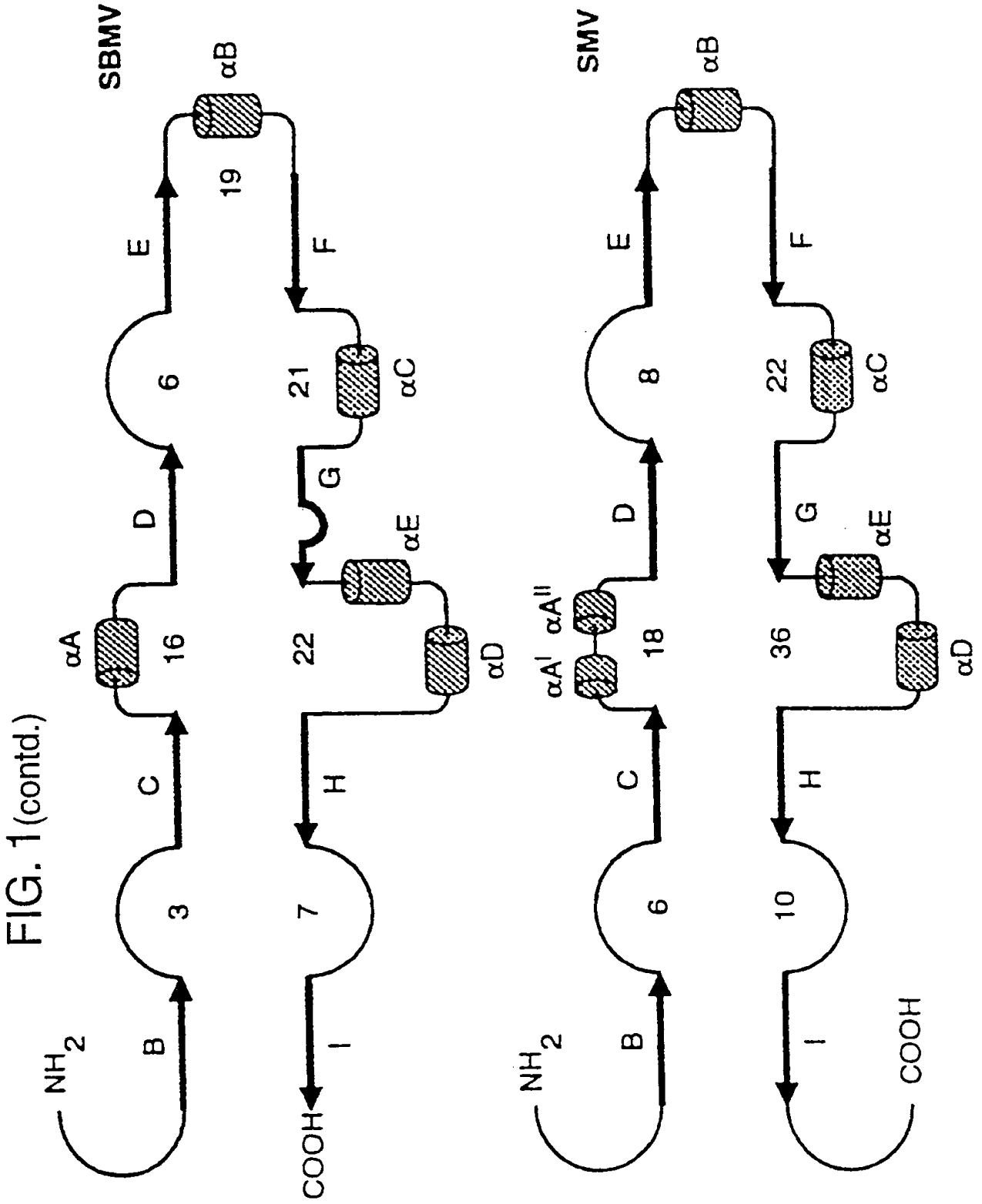
SBMV
αB
19
E
F
6
21
αC
D
G
αA
16
αE
22
αD
C
H
3
7
B
I
NH2
COOH
SMV
αB
E
F
8
22
αC
D
G
αA' αA''
18
αE
36
αD
C
H
6
10
B
I
NH2
COOH

FIG. 1(contd.)

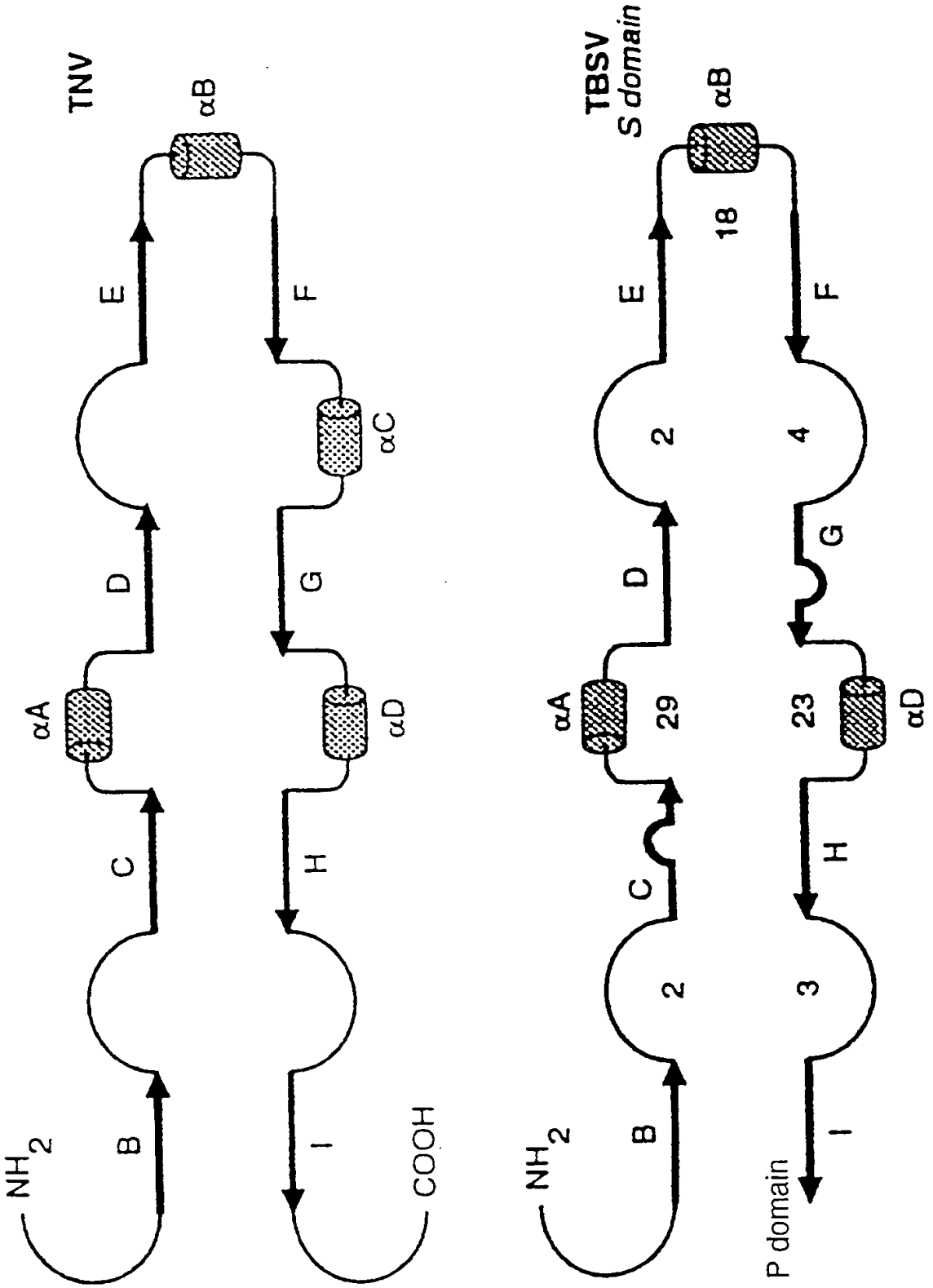
TNV
αB
E
F
αC
D
G
αA
αD
C
H
B
I
NH2
COOH
TBSV
S domain
αB
18
E
F
2
4
D
G
αA
29
23
αD
C
H
2
3
B
I
NH2
P domain

FIG. 1(contd.)

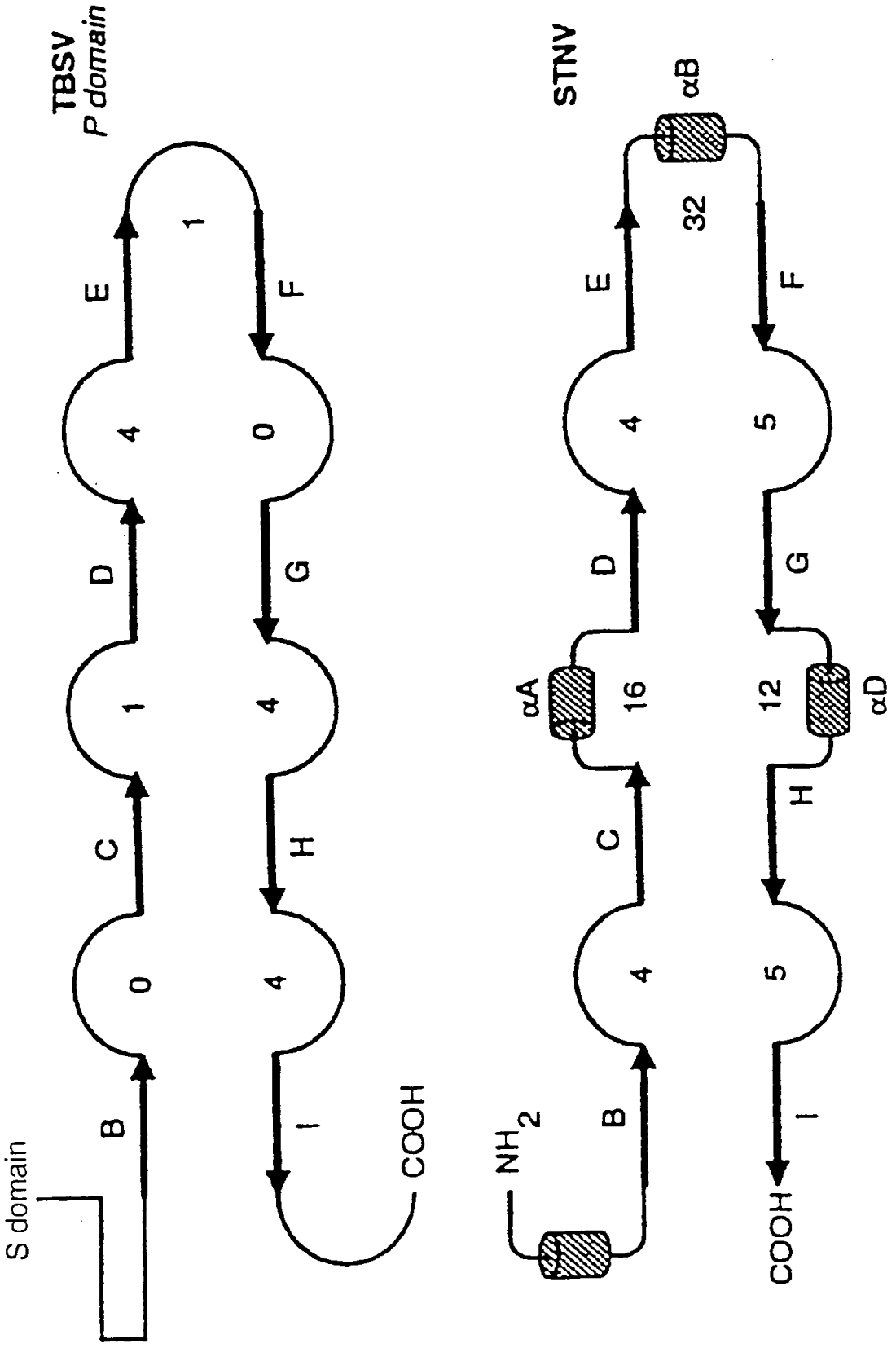
TBSV
P domain
S domain
B
0
C
1
D
4
E
1
F
0
G
4
H
4
I
COOH
STNV
αB
32
E
4
F
5
D
αA
16
G
12
αD
C
H
4
5
NH2
B
I
COOH

FIG. 1(contd.)

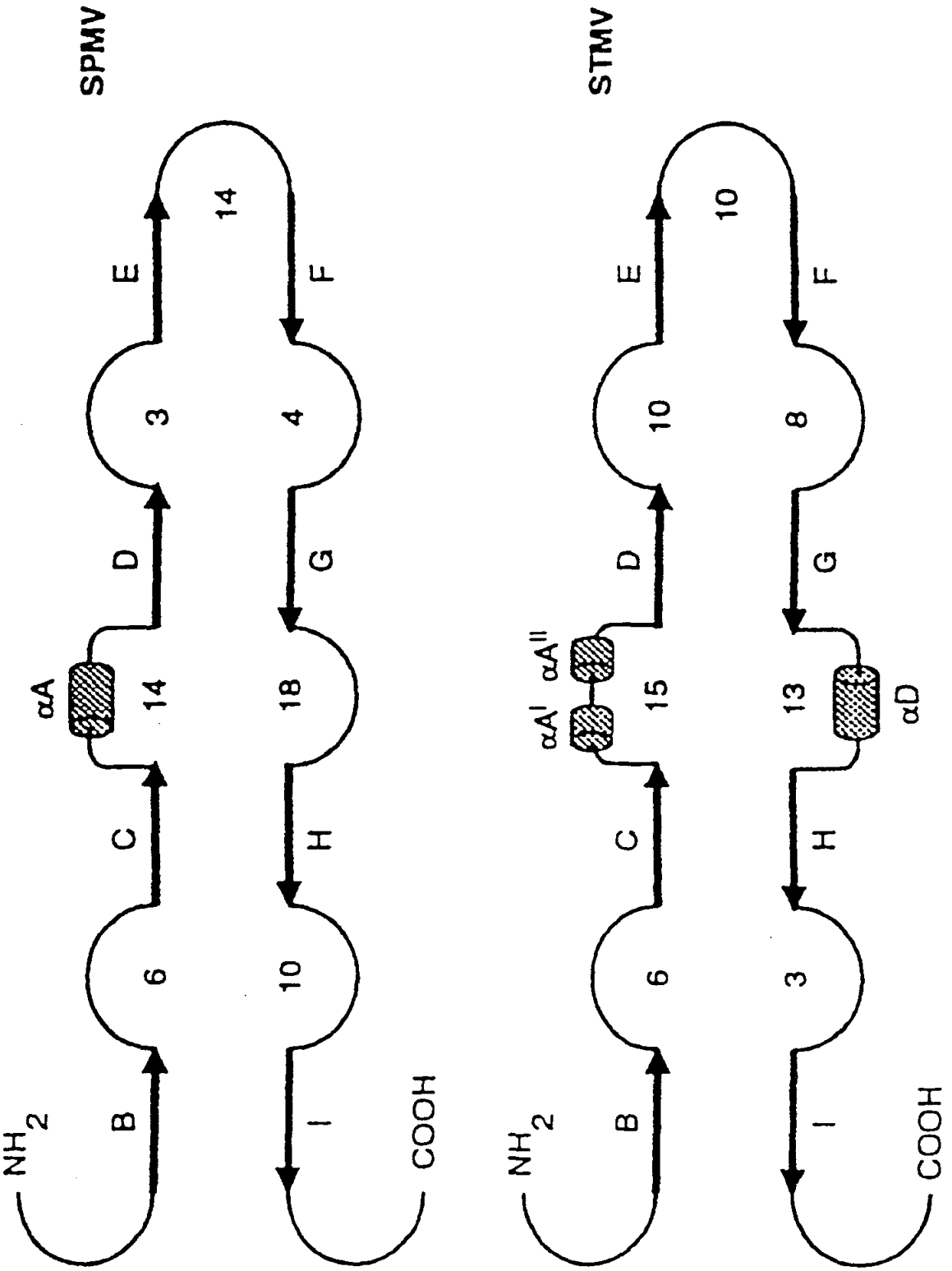

FIG. 2A

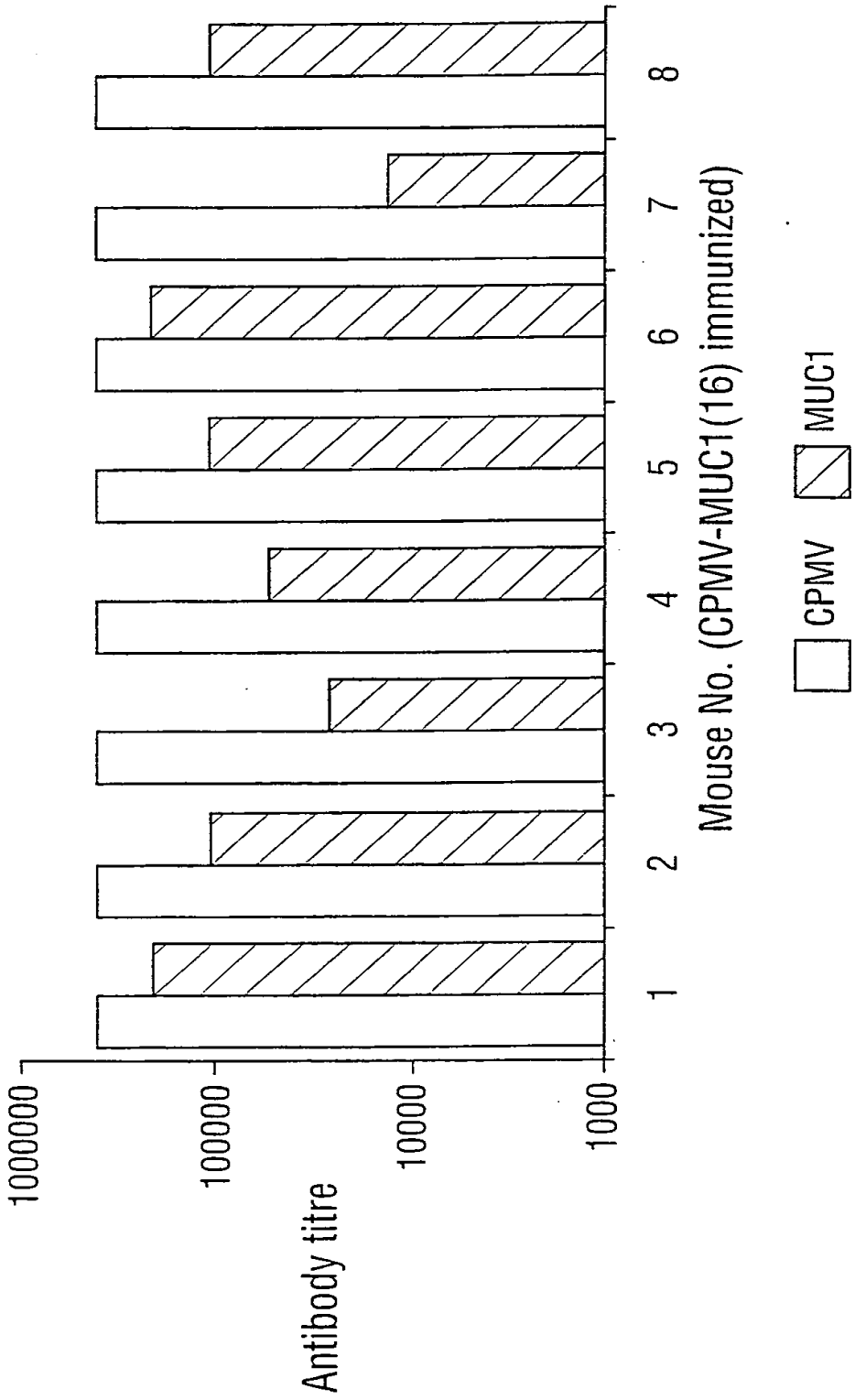
1000000
100000
10000
1000
Antibody titre
1
2
3
4
5
6
7
8
Mouse No. (CPMV-MUC1(16) immunized)
CPMV
MUC1

FIG. 2B

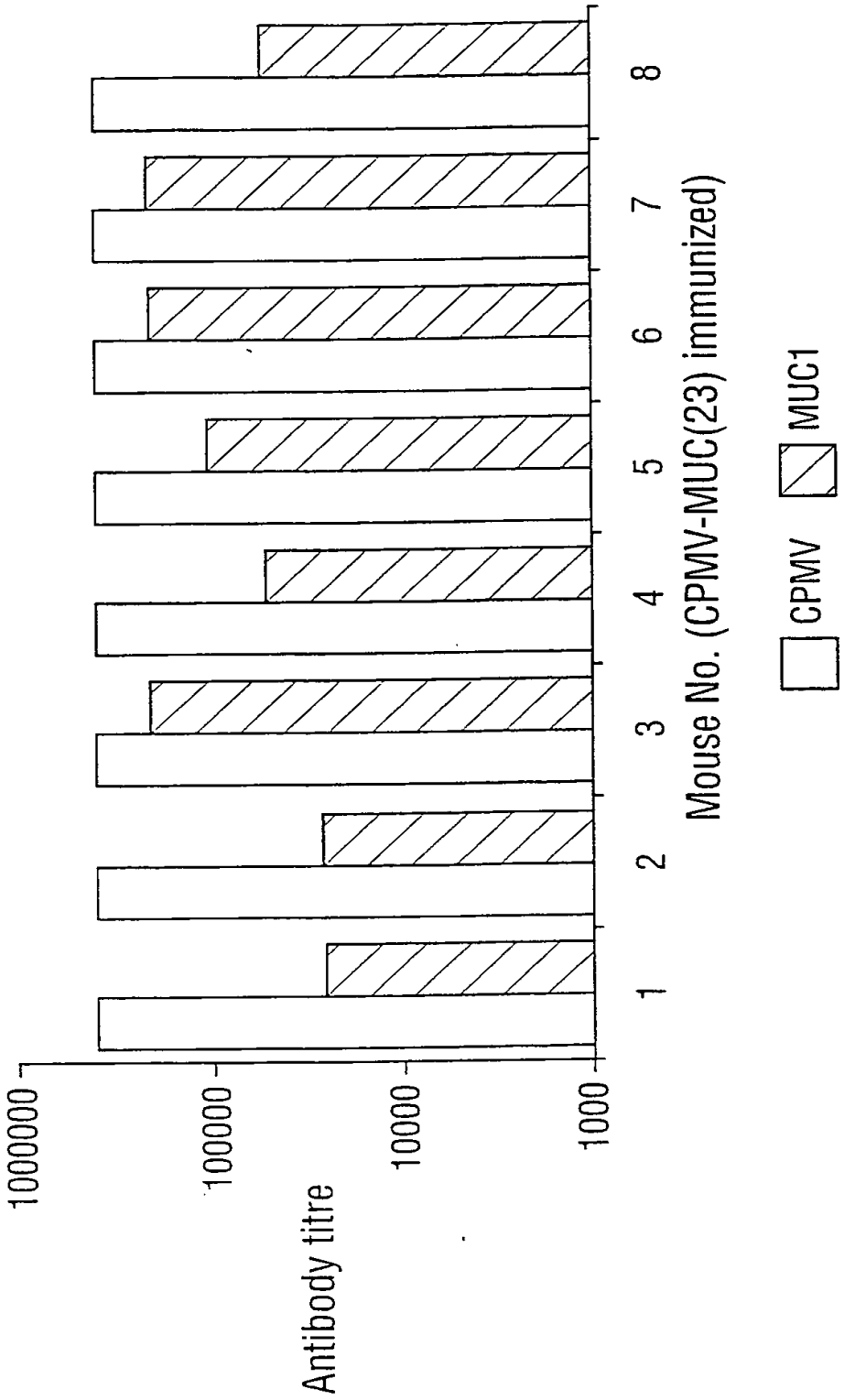
1000000
100000
10000
1000
Antibody titre
1
2
3
4
5
6
7
8
Mouse No. (CPMV-MUC(23) immunized)
CPMV
MUC1

FIG. 2C

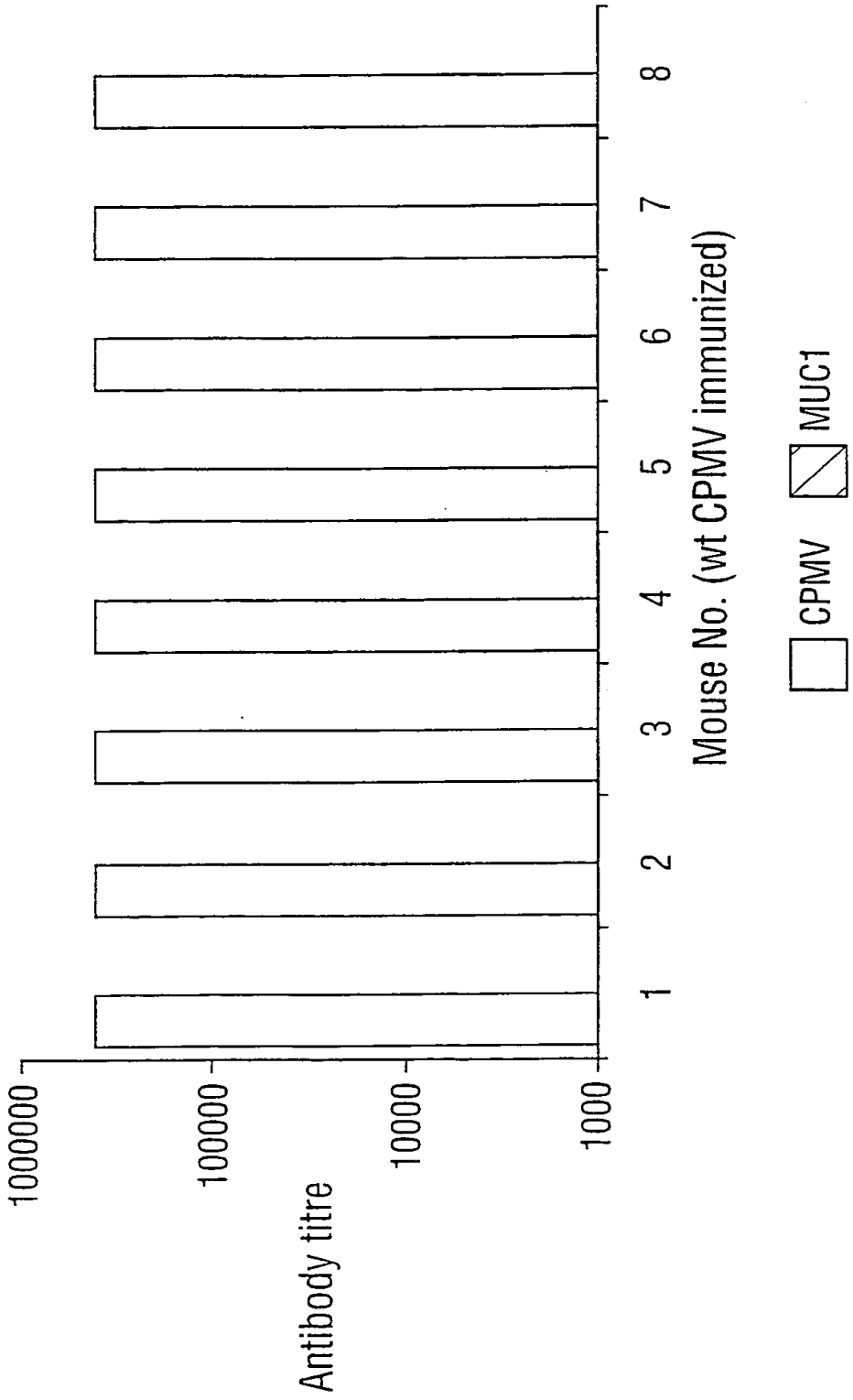
1000000
100000
10000
1000
Antibody titre
1
2
3
4
5
6
7
8
Mouse No. (wt CPMV immunized)
CPMV
MUC1

FIG. 3A

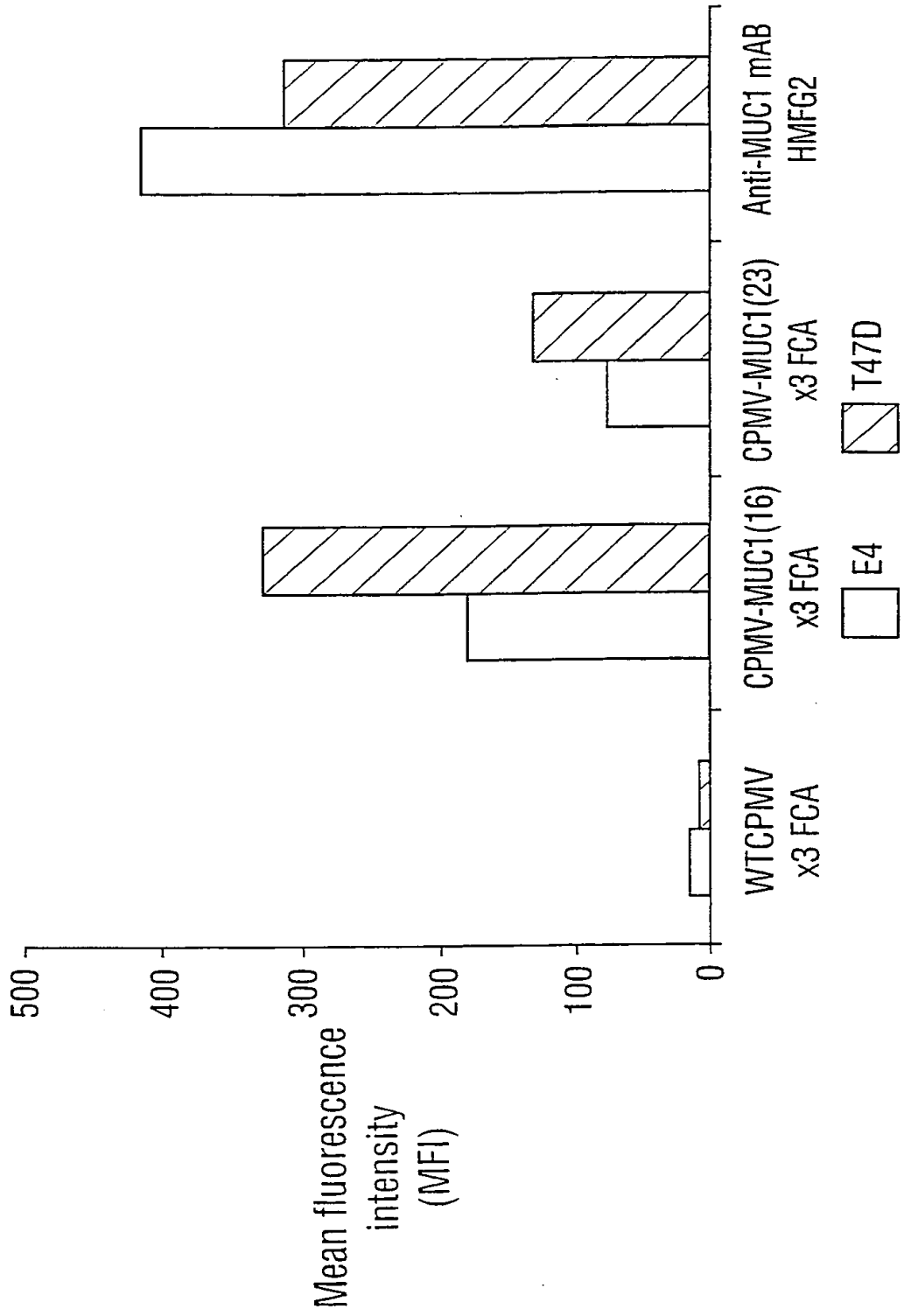
Mean fluorescence intensity (MFI)
500
400
300
200
100
0
WTCPMV x3 FCA
CPMV-MUC1(16) x3 FCA
CPMV-MUC1(23) x3 FCA
Anti-MUC1 mAB HMFG2
E4
T47D

FIG. 3B

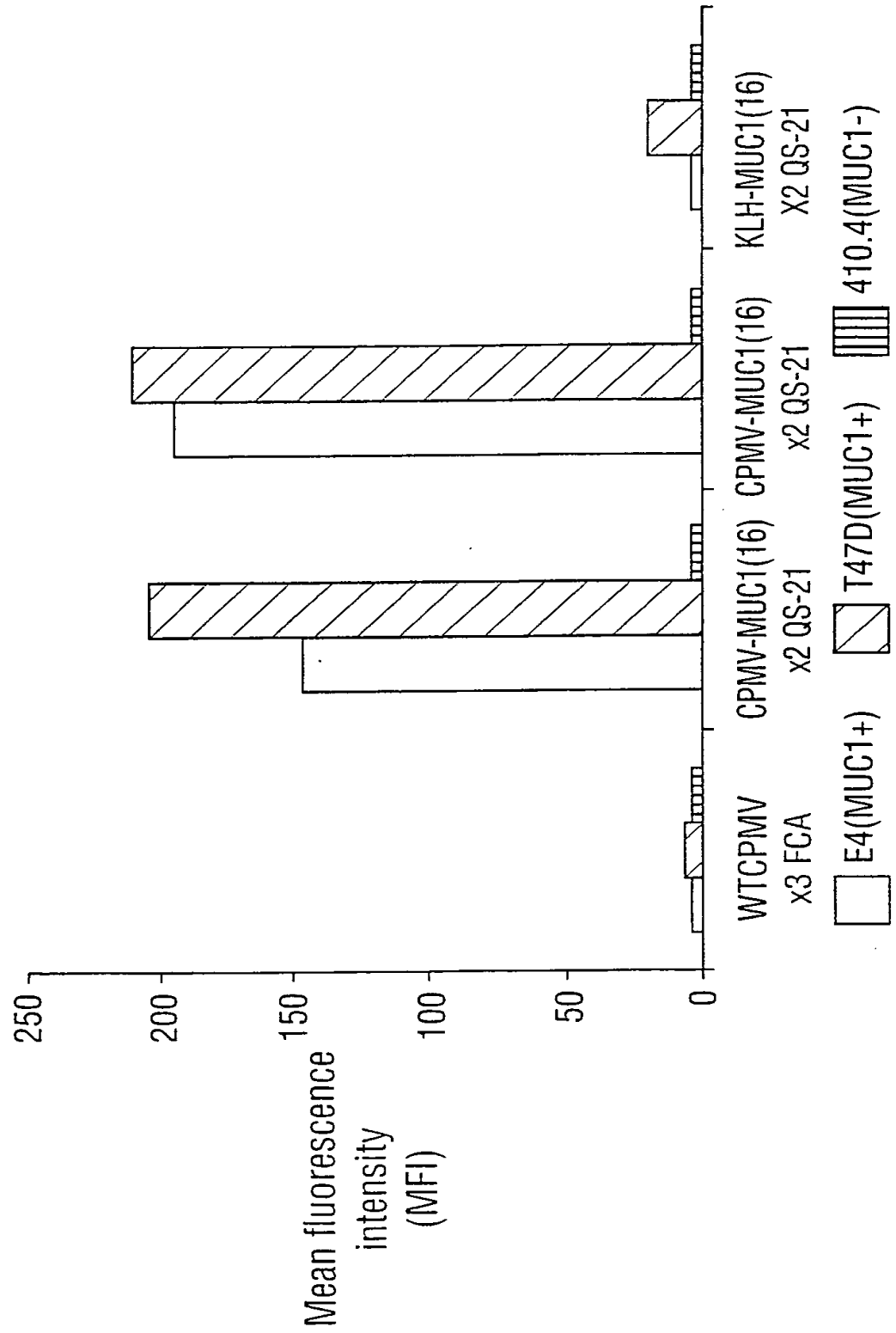
Mean fluorescence intensity (MFI)
250
200
150
100
50
0
WTCPMV x3 FCA
CPMV-MUC1(16) x2 QS-21
CPMV-MUC1(16) x2 QS-21
KLH-MUC1(16) X2 QS-21
E4(MUC1+)
T47D(MUC1+)
410.4(MUC1-)

FIG. 4

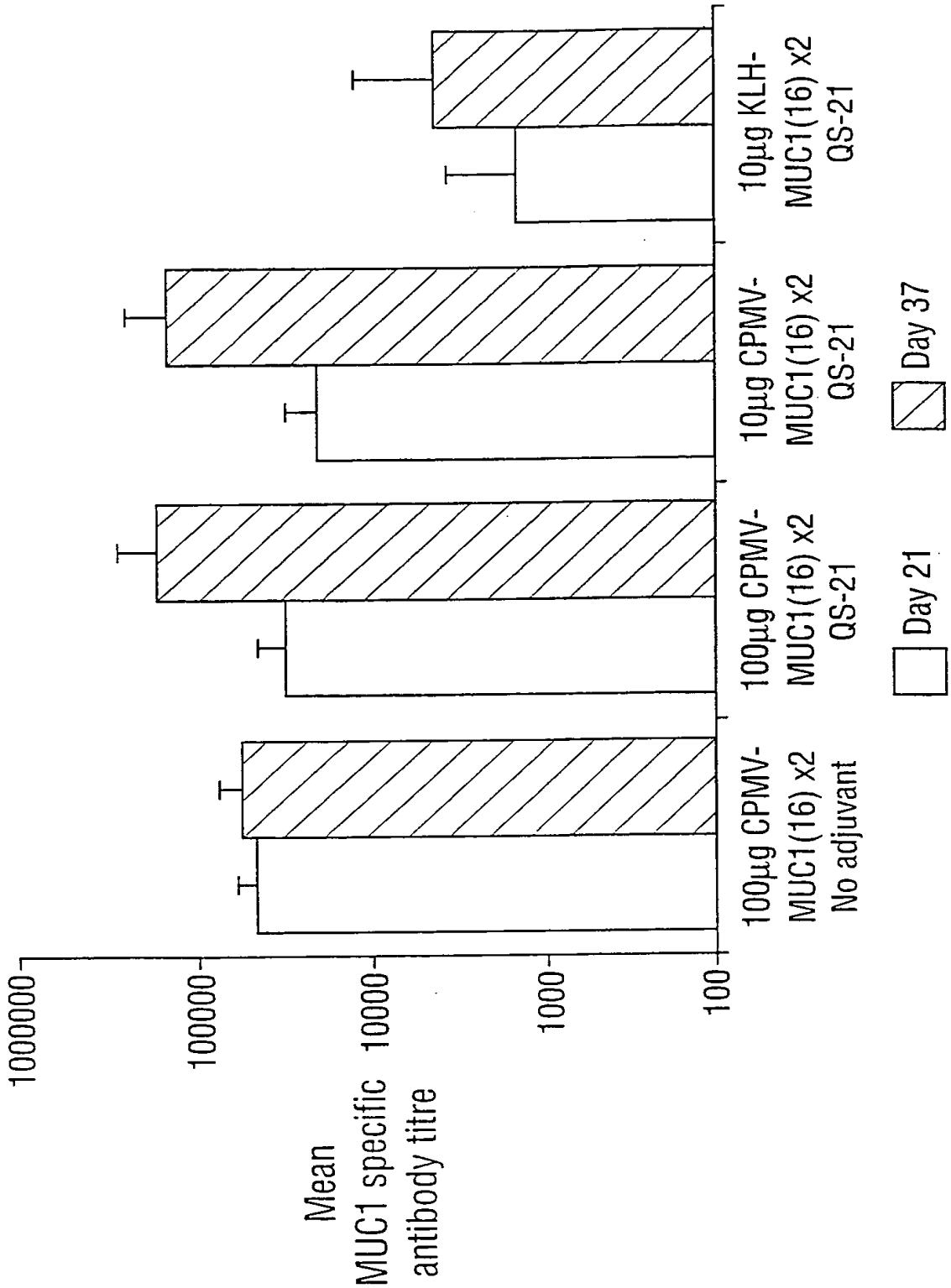
Mean MUC1 specific antibody titre
1000000
100000
10000
1000
100
100µg CPMV-MUC1(16) x2 No adjuvant
100µg CPMV-MUC1(16) x2 QS-21
10µg CPMV-MUC1(16) x2 QS-21
10µg KLH-MUC1(16) x2 QS-21
Day 21
Day 37

FIG. 5

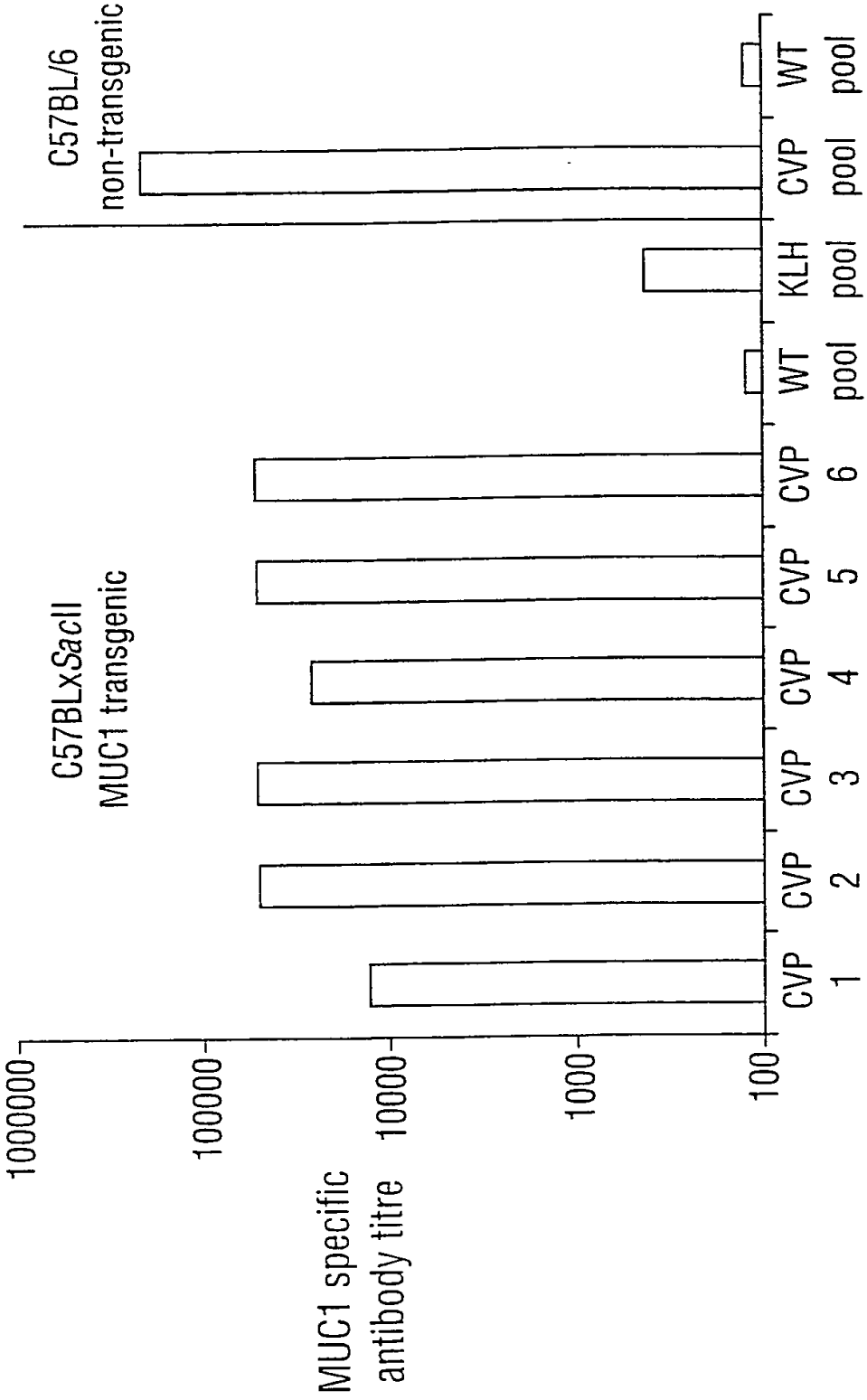
C57BL/6
non-transgenic
C57BLxSacII
MUC1 transgenic
MUC1 specific
antibody titre
1000000
100000
10000
1000
100
CVP
CVP
CVP
CVP
CVP
CVP
WT
KLH
WT
CVP
WT
1
2
3
4
5
6
pool
pool
pool
pool
pool

FIG. 6

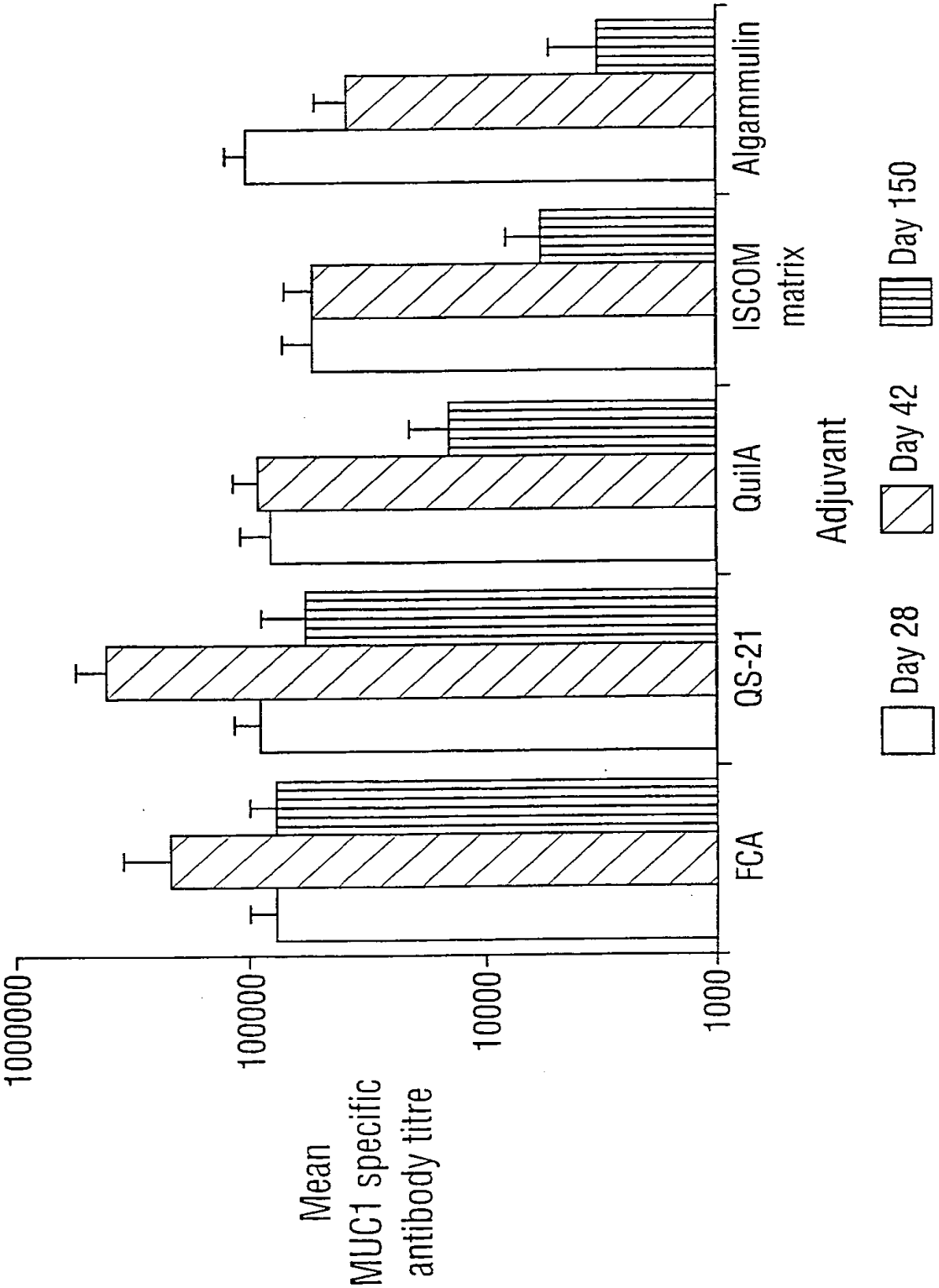
1000000
100000
10000
1000
Mean MUC1 specific antibody titre
FCA
QS-21
QuilA
ISCOM matrix
Algammulin
Adjuvant
Day 28
Day 42
Day 150

FIG. 7A

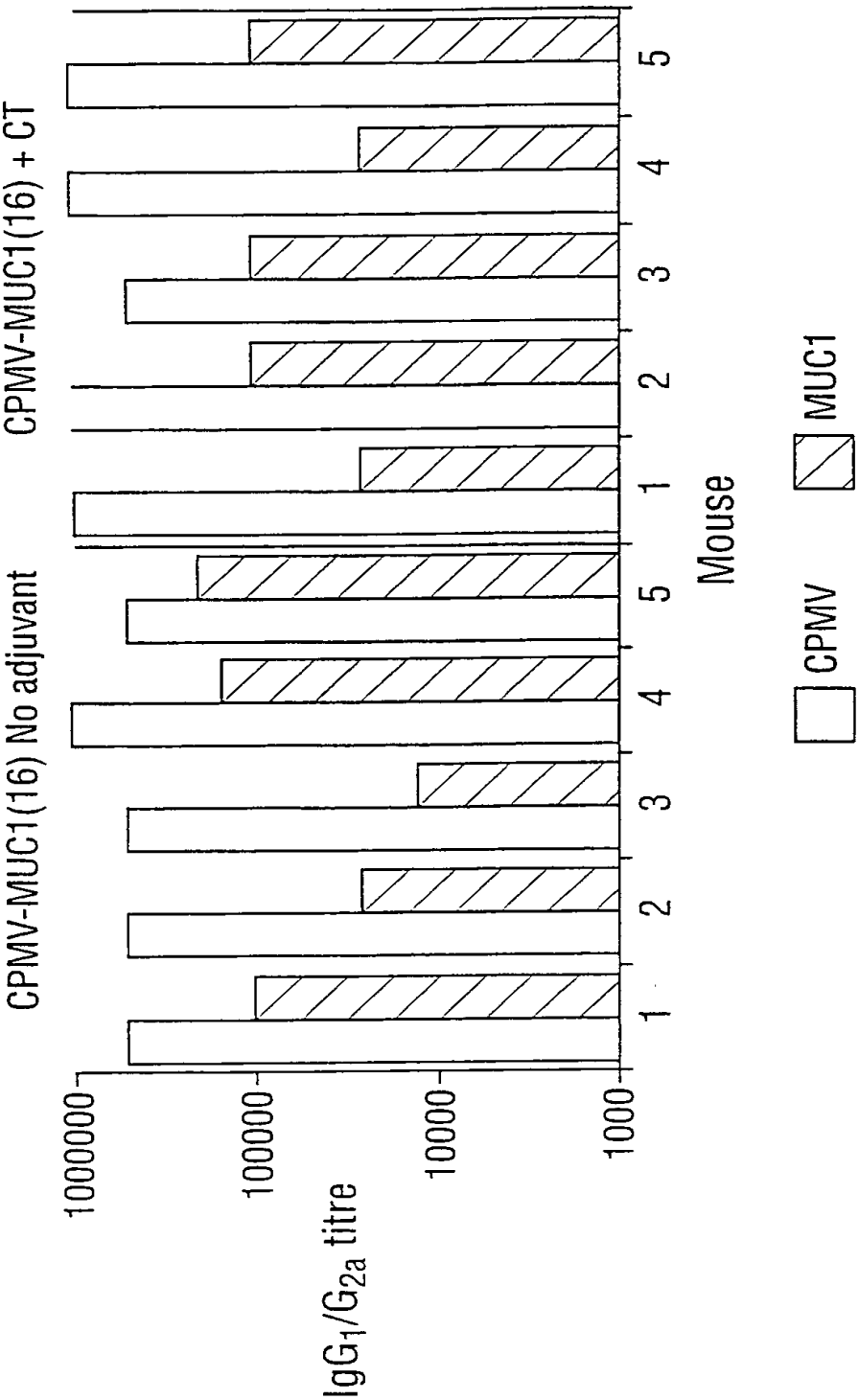
CPMV-MUC1(16) No adjuvant
CPMV-MUC1(16) + CT
1000000
100000
10000
1000
IgG1/G2a titre
1
2
3
4
5
Mouse
CPMV
MUC1

FIG. 7B

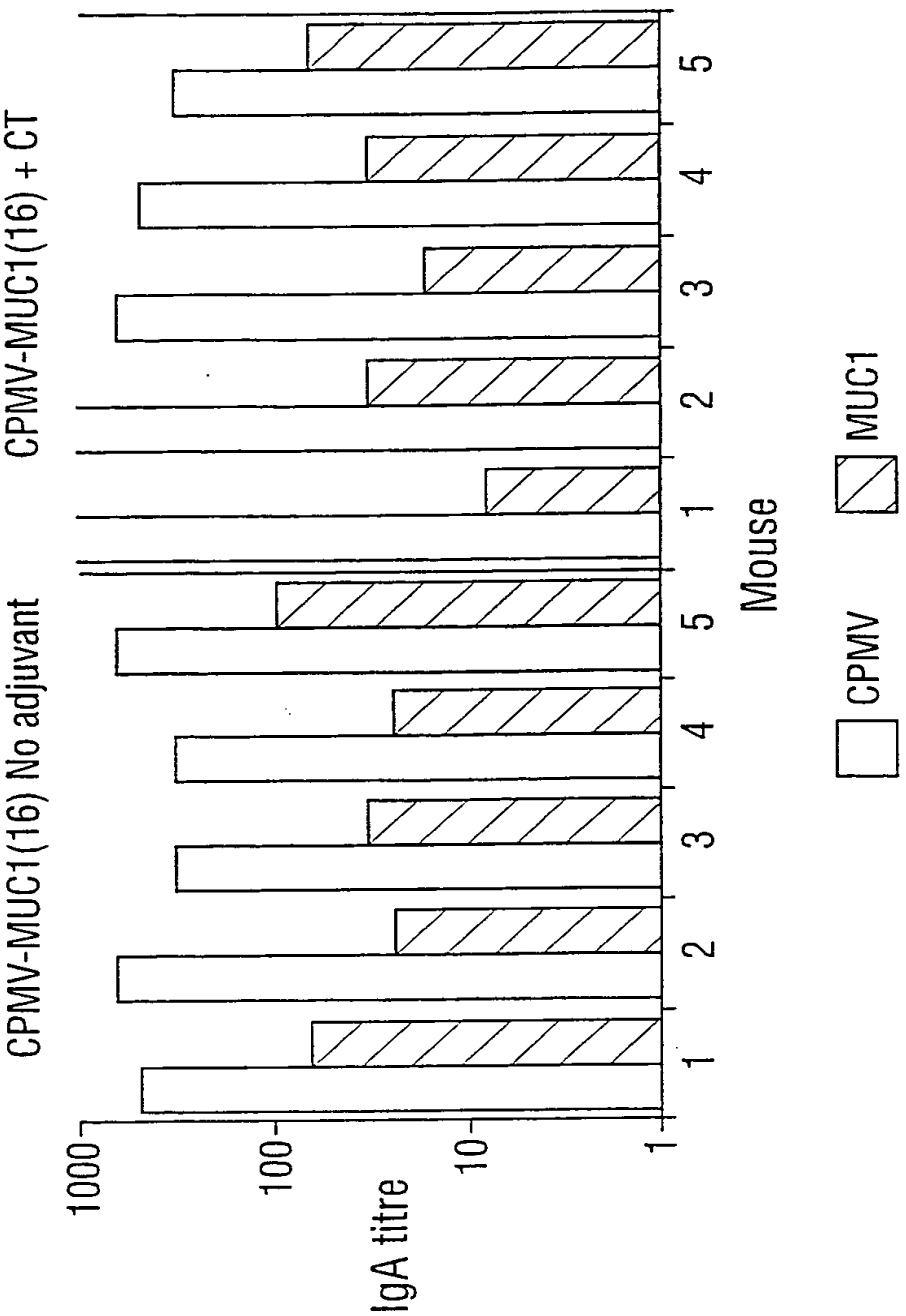
CPMV-MUC1(16) + CT
CPMV-MUC1(16) No adjuvant
1000
100
10
1
IgA titre
1
2
3
4
5
Mouse
CPMV
MUC1

FIG. 8(a)

Sequence of SBMV Coat Protein Spanning The Potential Insertion Site With Introduced Base Changes and New Restriction Sites: (sequence starts at nt 3955)

```
M  E  G  G  S  S  K  T  A  V  N  T  G
ATGGAAGGAGGATCATCTAAGACTGCTGTGAACACTGGG
             ↓                 ↓
          GGATCC            GTTAAC
          BamH I            Hpa I
```

FIG. 8(b)

Series of Sequences to be Inserted Between the Restriction Sites to Insert the MUC1(16) Epitope at Various Locations

```
G  V  T  S  A  P  D  T  R  P  A  P  G  S  T  A
GGTGTTACTTCTGCTCCTGATACTAGACCTGCTCCTGGTTCTACTGCT
CCACAATGAAGACGACCACTATGATCTGGACGAGGACCAAGATGACGA
```

GATCC	TCTAAGACTGCTGTT
G	AGATTCTGACGACAA
GATCCTCT	AAGACTGCTGTT
GAGA	TTCTGACGACAA
GATCCTCTAAG	ACTGCTGTT
GAGATTC	TGACGACAA
GATCCTCTAAGACT	GCTGTT
GAGATTCTGA	CGACAA
GATCCTCTAAGACTGCT	GTT
GAGATTCTGACGA	CAA

FIG. 9

```
LTSV  :  NI---YAPARLTIAA-ANSSINIASVGTLYATYEVEL
SBMV  :  NIGNILVPARLVIAMEGGSSKTAVNTGRLYASYTIRL
SMV   :  NIATDLVPARLVIALLDGSSSTAVAAGRIYASYTIQM
                   ######=======#############
                    βH     loop        βI
```

FIG. 13

```
                          220         230        240
         AA        |ASIVQKYVIDLGGTLTSFEGPSYLMPP
         PHD sec   | HHHHHEEEE   EEEE    EEEEE
         Rel sec   |145432244525515625586487624
detail :
         prH sec   |466655321111100000000000000
         prE sec   |101123456632246752212688753
         prL sec   |422221112246642237787311246
subset : SUB sec   |..H......E.LL.EE.LLLL.EEE..
```

Abbreviations :

- AA : amino acid sequence
- H : helix
- E : extended (sheet)
- blank : other (loop)
- PHD : Profile network prediction HeiDelberg
- Rel : Reliability index of prediction (0-9)
- prH : probability for assigning helix
- prE : probability for assigning strand
- prL : probability for assigning loop
- SUB : a subset of the prediction, for all residues with an average expected accuracy of >82%

FIG. 10(a)

Sequence of LTSV Coat Protein Spanning The Potential Insertion Site With Introduced Base Changes and New Restriction Sites: (sequence starts at nt 3954)

```
I  A  A  A  N  S  S  I  N  I  A  S  V  G  T  L  Y
ATAGCCGCAGCTAACAGCTCCATAAACATAGCTAGTGTGGGTACTCTTTAT
```

CTGCAG
Pst I

GGTACC
Kpn I

FIG. 10(b)

Series of Sequences to be Inserted Between the Restriction Sites to Insert the MUC1(16) Epitope at Various Locations.

```
G  V  T  S  A  P  D  T  R  P  A  P  G  S  T  A
GGTGTTACTTCTGCTCCTGATACTAGACCTGCTCCTGGTTCTACTGCT
CCACAATGAAGACGACCACTATGATCTGGACGAGGACCAAGATGACGA
```

GCTAACAGC	TCCATAAACATAGCTAGTGTGGGTAC
ACGTCGATTGTCG	AGGTATTTGTATCGATCACACC
GCTAACAGCTCC	ATAAACATAGCTAGTGTGGGTAC
ACGTCGATTGTCGAGG	TATTTGTATCGATCACACC
GCTAACAGCTCCATA	AACATAGCTAGTGTGGGTAC
ACGTCGATTGTCGAGGTAT	TTGTATCGATCACACC
GCTAACAGCTCCATAAAC	ATAGCTAGTGTGGGTAC
ACGTCGATTGTCGAGGTATTTG	TATCGATCACACC
GCTAACAGCTCCATAAACATA	GCTAGTGTGGGTAC
ACGTCGATTGTCGAGGTATTTGTAT	CGATCACACC
GCTAACAGCTCCATAAACATAGCT	AGTGTGGGTAC
ACGTCGATTGTCGAGGTATTTGTATCGA	TCACACC

FIG. 11

Lipman-Pearson alignment of RCNMV and TBSV coat protein sequences.

Lipman-Pearson Protein Alignment
Ktuple: 2; Gap Penalty: 4; Gap Length Penalty: 12

Seq1(1>389) tbsvtbs.PRO	Seq2(1>340) rcnmvdia.PRO	Similarity Index	Gap Number	Gap Length	Consensus Length
(64>387)	(8>338)	26.9	4	7	331

```
      ₣70        ₣80        ₣90        ₣100       ₣110       ₣120
KKQQMINHVGGTGGAIMAPVAVTRQLVGSKPKFTGRTSGSVTVTHREYLSQVNNSTGFQV
K.:Q. :. . T .: : .VA:. . .    . . ... : .: H : :  V .S. .:.
KSKQRSQPRNRTPNTSVKTVAIPFAKTQIIKTVNPPPKPARGILHTQLVMSVVGSVQMRT
  ₺10        ₺20        ₺30        ₺40        ₺50        ₺60
      ₣130       ₣140       ₣150       ₣160       ₣170       ₣180
NGGIVGNLLQLNPLNGTLFSWLPAIASNFDQYTFNSVVLHYVPLCSTTEVGRVAIYFDKD
N.G  .: ::LNP N :LF: L:  A:N:D Y ::.:.L:YVPL :. : GRVA: .D D
NNGKSNQRFRLNPSNPALFPTLAYEAANYDMYRLKKLTLRYVPLVTVQNSGRVAMIWDPD
  ₺70        ₺80        ₺90        ₺100       ₺110       ₺120
      ₣190       ₣200       ₣210       ₣220       ₣230       ₣240
SEDPEPADRVELANYSVLKETAPWAEAMLRVPTDKIKRFCDDSSTSDHKLIDLGQLGIAT
S:D:.P..R E::.YS   .TA ...  L :P:D:  RF .D::T D:KL:D:GQL :.T
SQDSAPQSRQEISAYSRSVSTAVYEKCSLTIPADNQWRFVADNTTVDRKLVDFGQLLFVT
  ₺130       ₺140       ₺150       ₺160       ₺170       ₺180
      ₣250       ₣260       ₣270       ₣280       ₣290            ₣300
YGGAGTNAVGDIFISYSVTLYFPQPTNTLLSTRRLDLAGALVTASGPGYLLVSR---TAT
.:G::. ..GDIF:...V.:  PQPT.:::  .   :DL:G:L.: .GP:YL: :    T::
HSGSDGIETGDIFLDCEVEFKGPQPTASIVQKTVIDLGGTLTSFEGPSYLMPPDAFITSS
  ₺190       ₺200       ₺210       ₺220       ₺230       ₺240
         ₣310       ₣320       ₣330       ₣340       ₣350
VLTMTFRATGTFVISGTYRCLTATTLGLAG--GVNVNSITVVDNIG-TDSAFFINCTVSN
 :.:   .:GT:::: .  C T:.:.::G  .:  :: :. :: . ..S  F.:..V :
SFGLFVQVAGTYLLTLVVTCSTTGSVTVGGNSTLVGDGRAAYGSSNYIASIVFTSSGVLS
  ₺250       ₺260       ₺270       ₺280       ₺290       ₺300
  ₣360       ₣370       ₣380
LPSVVTFT-STGITSATVHCVRATRQNDVSL
 .: V F: S:G::.. ::  R .: N.  L
TTPSVQFSGSSGVSRVQMNICRCKQGNTFIL
  ₺310       ₺320       ₺330
```

FIG. 12

Beta plot - Chou-Fasman

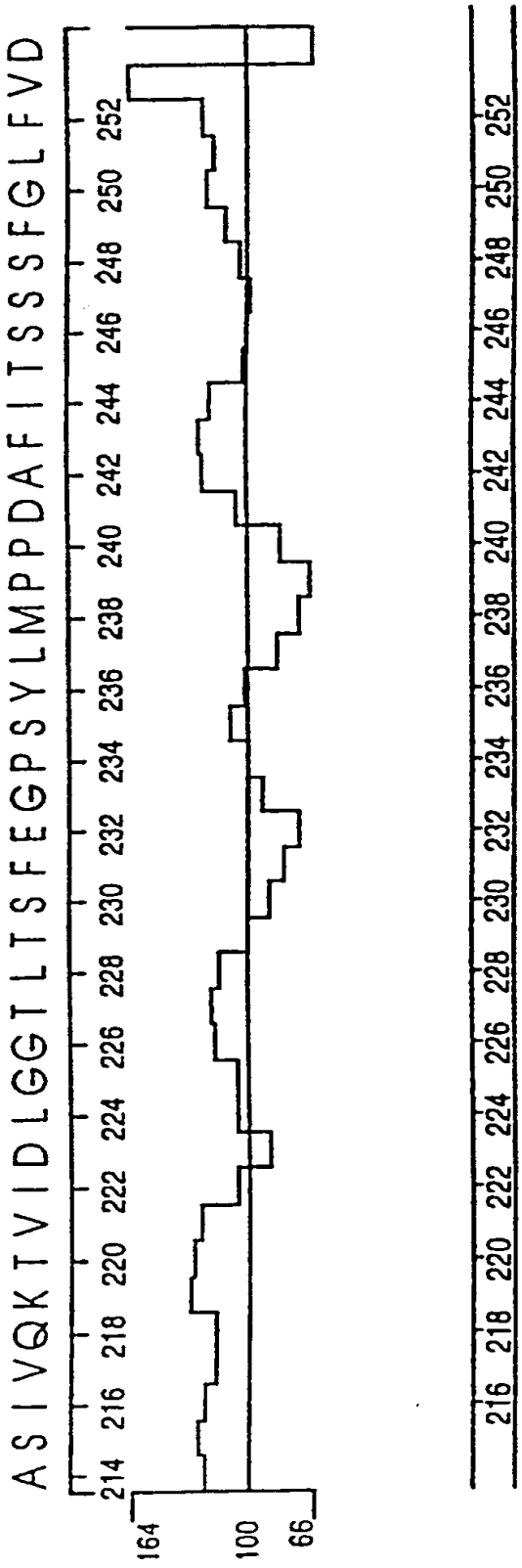

FIG. 14(a)

Sequence of RCNMV Coat Protein Spanning The Potential Insertion Site With Introduced Base Changes and New Restriction Sites: (sequence starts at nt 3070)

S I V Q K T V I D L G G T L T S F

AGCATCGTACAGAAAACTGTAATTGATCTCGGTGGGACACTCACTTCTTTC

GTGCAC
*Apa*LI

GTTAAC
*Hpa*I

FIG. 14(b)

Series of Sequences to be Inserted Between the Restriction Sites to Insert the MUC1(16) Epitope at Various Locations

G V T S A P D T R P A P G S T A

GGTGTTACTTCTGCTCCTGATACTAGACCTGCTCCTGGTTCTACTGCT

CCACAATGAAGACGACCACTATGATCTGGACGAGGACCAAGATGACGA

GAAAACTGTA	ATTGATCTCGGTGGGACGTT
ACGTCTTTTGACAT	TAACTAGAGCCACCCTGCAA
GAAAACTGTAATT	GATCTCGGTGGGACGTT
ACGTCTTTTGACATTAA	CTAGAGCCACCCTGCAA
GAAAACTGTAATTGAT	CTCGGTGGGACGTT
ACGTCTTTTGACATTAACTA	GAGCCACCCTGCAA
GAAAACTGTAATTGATCTC	GGTGGGACGTT
ACGTCTTTTGACATTAACTAGAG	CCACCCTGCAA
GAAAACTGTAATTGATCTCGGT	GGGACGTT
ACGTCTTTTGACATTAACTAGAGCCA	CCCTGCAA
GAAAACTGTAATTGATCTCGGTGGG	ACGTT
ACGTCTTTTGACATTAACTAGAGCCACCC	TGCAA